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Levels and Patterns of Genetic Variation in *Isoëtes karstenii* with Observations on *I. palmeri*

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ABSTRACT.—Genetic variation was surveyed in eleven populations from throughout the range of the diploid *Isoëtes karstenii,* as well as in two populations of a closely related tetraploid, *I. palmeri,* using allozyme electrophoresis. Genetic variation was high in *I. karstenii* and appears to be highly structured, as evidenced by analyses of F-statistics and genetic vs. geographic distance. No fixed unique alleles were found that could distinguish between *I. karstenii* and *I. palmeri,* although one allele was observed that was unique to *I. palmeri.* These analyses confirm earlier observations suggesting low levels of interpopulational gene flow and have implications for sampling strategies.

Isoëtes karstenii A. Braun was first described based on a collection from the high-altitude páramos of Merida, Venezuela (Braun, 1862). Since that time it has variously been accepted or synonymized by subsequent researchers, yet no detailed discussions of its variation or distribution have been published. As part of continuing research aimed at characterizing the neotropical species of *Isoëtes,* we performed a survey of genetic variation in *I. karstenii* from throughout its range, as well as in a pair of populations from a single locality of a related species, *I. palmeri* H.P. Fuchs, using allozyme electrophoresis. A taxonomic revision of the species complex to which these taxa belong is currently in preparation (Small and Hickey, in prep.).

The utility of allozyme data in plant systematics in general is well documented (Gottlieb, 1977, 1982; Crawford, 1985; Weeden and Wendel, 1989), and has been applied profitably to studies of *Isoëtes* (Taylor et al., 1985; Hickey et al., 1989a,b; Duff and Evans, 1992). In *Isoëtes,* allozymes have been useful in delimiting species, assessing parentage of polyploid taxa and hybrids, and hypothesizing phylogenetic relationships within species complexes.

MATERIALS AND METHODS

Materials and methods for enzyme electrophoresis follow standard protocols derived from published electrophoretic and staining procedures (Soltis et al., 1983; Werth, 1985). Individual specimens from eleven populations from throughout the range of the diploid *I. karstenii* and two populations of the tetraploid *I. palmeri* (Small, 1994) were included in the analyses (see Table 1 for locality, number of individuals sampled, and voucher information). Individuals subjected to electrophoresis were grown in a cold room at ca. 4°C under a 16:8 hr light:dark cycle, fully submerged, and potted in fine quartz sand or in a mixture of 2 parts sphagnum peat: 3 parts quartz sand, as sug-

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gested by Taylor and Luebke (1986). Samples for electrophoresis consisted of leaf tips ca. 1–2 cm long that were collected either the night before or immediately prior to electrophoresis; no differences were detected between samples collected the night before and those collected immediately prior to electrophoresis. Leaf samples were ground over ice in "microbuffer" (Werth, 1985, Appendix 1), and the resulting homogenate absorbed into wicks of Whatman 3 MM filter paper that were inserted into starch gels (15% w:v). Ten enzyme systems were resolved using five different buffer systems (several enzymes were stained on multiple buffer systems): glutamate dehydrogenase (GDH), 6-phosphogluconate dehydrogenase (6PGDH), phosphoglucoisomerase (PGI), and shikimate dehydrogenase (SKDH) were resolved on a Tris-citrate pH 8.0 buffer (Werth, 1985); isocitrate dehydrogenase (IDH), 6PGDH, phosphoglucomutase (PGM), SKDH, and triosephosphate isomerase (TPI) were resolved on buffer system 5 (Soltis et al., 1983); IDH was resolved on buffer system 11 (Soltis et al., 1983); leucine aminopeptidase (LAP), TPI, and non-specific protein (NSP; i.e., RUBISCO, Wendel and Weeden, 1989) were resolved on a LiOH buffer system (Werth, 1985). Allozyme phenotypes were documented by photographing the gel slices when fully developed. Assignment of allelic designations to individuals run on different gels was performed both by including a sample from a control plant on each gel (*I. flaccida* A. Braun), and by running samples from different gels with the same putative alleles on a "lineup" gel.

Allozyme phenotypes were scored for each individual at twelve loci and interpreted genetically according to standard allozyme analysis based on known quaternary structure and cellular compartmentalization (Weeden and Wendel, 1989) and past experience with interpretation of *Isoëtes* allozymes (Hickey et al., 1989a,b; Taylor and Hickey, 1992). The most anodally migrating isozyme was arbitrarily designated as locus one, the next most anodally migrating, as locus two, and likewise, the most anodally migrating allozyme at each locus was arbitrarily assigned the designation *a,* the next most anodally migrating allozyme *b,* etc. Genotype frequencies (allele frequencies for *I. palmeri*) for each population were calculated and analyzed using the BIOSYS-1 software package (Swofford and Selander, 1981). Measures of genetic distance of Nei (1972, 1978) and Rogers (1972), and genetic similarities of Nei (1972, 1978) and Rogers (1972) were computed from the allele frequency data and distance matrices constructed. Measures of genetic variability (number of alleles per locus, percentage of polymorphic loci) and F-statistics were calculated. Phenograms of population relationships were constructed by the unweighted pair group method with arithmetic averaging (UPGMA; Sneath and Sokal, 1973) using the genetic distance and similarity measures previously calculated. Additionally, for *I. karstenii,* geographic distance vs. genetic distance was plotted for all pairwise comparisons of populations and a correlation coefficient calculated.

RESULTS

In *I. karstenii,* of the ten enzymes scored, three (GDH, LAP and NSP) were represented by a single locus each and were monomorphic across all popu-

TABLE 1. Population designations, species, location of collection and voucher information for populations included in this study. The number in parentheses following the population designation indicates the number of individuals sampled from that population.

Population Designation	Species	Collection Location	Voucher & Location
S-4 (40)	*I. karstenii*	Merida, Venezuela	*Small 4 & Hickey,* MU
S-5 (40)	*I. karstenii*	Merida, Venezuela	*Small 5 & Hickey,* MU
S-6 (40)	*I. karstenii*	Merida, Venezuela	*Small 6 & Hickey,* MU
S-7 (40)	*I. karstenii*	Merida, Venezuela	*Small 7 & Hickey,* MU
S-8 (40)	*I. karstenii*	Merida, Venezuela	*Small 8 & Hickey,* MU
S-126 (20)	*I. karstenii*	Boyaca, Colombia	*Small 126 & Gonzalez,* MU, COL
S-127 (20)	*I. karstenii*	Boyaca, Colombia	*Small 127 & Gonzalez,* MU, COL
S-128 (20)	*I. karstenii*	Boyaca, Colombia	*Small 128 & Gonzalez,* MU, COL
			Small 129 & Gonzalez, MU, COL
S-141 (20)	*I. karstenii*	Boyaca, Colombia	*Small 141 & Gonzalez,* MU, COL
S-144 (20)	*I. karstenii*	Boyaca, Colombia	*Small 144 & Gonzalez,* MU, COL
J-79 (4)	*I. karstenii*	Cundinamarca, Colombia	*Jermy 17479,* BM
S-155 (20)	*I. palmeri*	Cundinamarca, Colombia	*Small 155 & Gonzalez,* MU, COL
			Small 156 & Gonzalez, MU, COL
			Small 157 & Gonzalez, MU, COL
J-77 (28)	*I. palmeri*	Cundinamarca, Colombia	*Jermy 17477,* BM
			Jermy 17478, BM
			Jermy 17480, BM
			Jermy 17481, BM
			Jermy 17482, BM

lations. The remaining seven enzymes, scored for nine putative loci, showed variation in at least one population. Several populations contained unique alleles in low frequencies, but no fixed, unique alleles were observed in any population. Allele frequencies for all populations are shown in Table 2 and a pairwise distance matrix (Nei's [1978] unbiased genetic distance) is shown in Table 3. Characterization of each polymorphic locus scored is described below. Population designations follow Table 1.

IDH.—This enzyme was coded by a single locus. All populations were monomorphic for allele *b* at this locus except for S-127 and S-128. The populations S-127 and S-128 expressed alleles *a* and *b,* with *a* being the most common (frequency 0.775) in S-127, and *b* being the most common (frequency 0.925) in S-128.

MDH.—This enzyme exhibited an exceedingly complicated banding pattern. Two putative loci were scored for this enzyme. The first was represented by a group of the most anodally migrating bands. These bands always comigrated in a constant pattern and showed additivity in putative heterozygotes. Two alleles were detected at this locus with allele *a* being the most common in all populations. Alleles *a* and *b* were present in all Venezuelan populations (except S-6, which was fixed for *a*) whereas all Colombian populations were monomorphic for allele *a*. The second locus scored consisted of a group of more cathodally migrating bands. This locus was uninterpretable in regard to

TABLE 2. Estimated allele frequencies for all populations sampled. Population designations and sample sizes follow Table 1.

	Population												
Locus	S-4	S-5	S-6	S-7	S-8	S-126	S-127	S-128	S-141	S-144	J-79	S-155	J-77
Gdh													
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh													
a							0.775	0.075				0.500	0.446
b	1.000	1.000	1.000	1.000	1.000	1.000	0.225	0.925	1.000	1.000	1.000	0.325	0.250
c												0.175	0.304
Lap													
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-1													
a	0.875	0.975	1.000	0.550	0.900	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	0.125	0.025		0.450	0.100								
Mdh-2													
a	0.950	1.000	1.000	1.000	1.000								
b	0.050					1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6-Pgdh													
a	0.925	0.950	1.000	0.700	0.475	1.000	1.000	0.925	0.350	1.000			
b	0.075	0.050		0.300	0.525			0.075	0.650				0.304
c											1.000	1.000	0.696
Pgi													
a						0.425	0.600	0.075				0.500	1.000
b	1.000	1.000	1.000	1.000	1.000	0.550	0.400	0.925	1.000	1.000	1.000	0.500	
c						0.025							
Pgm													
a	0.100	0.725	0.850	0.184	0.363	1.000	1.000	0.925	0.725	0.500	0.500	0.500	0.571
b	0.900	0.275	0.150	0.816	0.367			0.075	0.275	0.500		0.400	
c											0.500	0.100	0.429
Skdh													
a								0.075					
b	1.000	0.837	1.000	1.000	1.000	0.500	0.475	0.400	0.475	0.300	1.000	0.500	0.625
c		0.162				0.500	0.525	0.525	0.525	0.700		0.500	0.375
Tpi-1													
a						0.025						0.071	
b	1.000	1.000	1.000	0.837	0.975	0.975	1.000	0.975	1.000	1.000	1.000	0.929	1.000
c				0.163	0.025			0.025					
Tpi-2													
a											0.125		
b	0.050					1.000	1.000	0.925	0.650	0.500	0.875	0.500	0.464
c	0.712	0.800	0.837	0.600	0.538			0.075	0.350	0.500			
d	0.238	0.200	0.163	0.400	0.463							0.500	0.536
Nsp													
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

allelic variation, due to a complicated multi-banded pattern that was present in some individuals, but not in others. It was therefore scored conservatively as present or absent ("allele" *a* = present, "allele" *b* = absent). This locus was present in all individuals of all Venezuelan populations (with the exception

TABLE 3. Table of Nei's (1978) unbiased genetic distance for all populations sampled. Population designations follow Table 1.

Population	S-4	S-5	S-6	S-7	S-8	S-126	S-127	S-128	S-141	S-144	J-79	S-155
S-4	—											
S-5	0.028	—										
S-6	0.053	0.009	—									
S-7	0.032	0.066	0.094	—								
S-8	0.029	0.033	0.048	0.017	—							
S-126	0.322	0.243	0.239	0.395	0.313	—						
S-127	0.405	0.317	0.313	0.493	0.399	0.055	—					
S-128	0.203	0.123	0.128	0.261	0.201	0.107	0.158	—				
S-141	0.322	0.264	0.279	0.408	0.342	0.039	0.106	0.120	—			
S-144	0.283	0.250	0.280	0.372	0.322	0.070	0.139	0.119	0.004	—		
J-79	0.459	0.438	0.422	0.486	0.414	0.166	0.249	0.325	0.156	0.178	—	
S-155	0.395	0.371	0.401	0.414	0.349	0.191	0.158	0.241	0.199	0.204	0.114	—
J-77	0.531	0.477	0.482	0.525	0.436	0.206	0.180	0.310	0.172	0.190	0.080	0.050

of two individuals in S-4) and absent from all individuals of all Colombian populations.

6-PGDH.—This enzyme was scored for one locus. An additional zone of activity was observed, but not consistently, and so was not scored. Three alleles were detected at this locus; allele *a* was observed in the highest frequency in all populations except S-8, S-141 and J-79. Populations S-8 and S-141 had allele *b* as the most common allele, but J-79 was the only *I. karstenii* population to exhibit allele *c*.

PGI.—This enzyme was scored for one locus. Again, a second zone of banding was detected, but inconsistently, so was not scored. Three alleles were detected at this locus. Populations S-4 through S-8 (Venezuelan populations) were monomorphic for allele *b*. The Colombian populations all exhibited both alleles *a* and *b* or were fixed for allele *a*, except that S-126 had allele *c* in low frequency (0.025) in addition to alleles *a* and *b*.

PGM.—This enzyme was represented by a single locus with three alleles detected. In populations S-4 through S-144 only alleles *a* and/or *b* were detected in varying combinations. Allele *c* was detected only in population J-79 in conjunction with allele *a*.

SKDH.—This enzyme was represented by a single locus with three alleles detected. All populations were either fixed for allele *b* or had a combination of alleles *b* and *c*, except that S-128 exhibited allele *a* at low frequency (0.075).

TPI.—This enzyme was represented by two loci. A third zone of banding representing post-translational modification of locus two (Hickey et al., 1989a) was also observed. *Tpi-1* was relatively uniform across all populations. Allele *b* was by far the most common allele; three populations (S-7, S-8, and S-128) also exhibited allele *c* in low frequency, and one population (S-126) exhibited

allele *a* in low frequency. *Tpi-2* was a highly variable locus, exhibiting the highest number of alleles (four) in this study. All populations exhibited at least two alleles at this locus except that S-126 and S-127 were fixed for allele *b*.

In addition to the populations of *I. karstenii* surveyed, two populations of *I. palmeri,* a closely related tetraploid (Small, 1994) also were surveyed. These collections were made by two different collectors (R. Small and A. C. Jermy) in different years at the same locality (vicinity of Laguna Chisaca, Dpto. Cundinamarca, Colombia), which consists of a large lake and many surrounding pools. The S-155 and J-77 populations each consist of multiple collections from different sites within a single pool; the two populations, however, are clearly from different pools (A. C. Jermy, pers. comm.).

Isoëtes palmeri, like *I. karstenii,* was also monomorphic for isozymes GDH, LAP, and NSP and in addition was monomorphic for allele *a* at *Mdh-1,* and for allele *b* at *Mdh-2* (absence of the locus). The remaining loci exhibited two or three alleles. One allele was detected that was unique to, but not fixed, in *I. palmeri* relative to *I. karstenii*: *Idh* allele *c,* frequency = 0.341. These populations exhibited patterns of apparent fixed heterozygosity at some loci, as might be expected in an allotetraploid (Werth, 1989). Interestingly, however, patterns of fixed heterozygosity were polymorphic within these populations. For example, in the population S-155, individuals were collected at three sites within a pool of approximately 100 m^2 (corresponding to *Small & Gonzalez 155, 156* & *157* of Table 1). At the locus *Skdh,* all individuals showed the *bc* heterozygous phenotype; for *Idh,* however, the subpopulations 155 and 156 had an *ab* heterozygous phenotype, whereas the subpopulation 157 showed an *ac* heterozygous phenotype. Similarly, at *Pgm* subpopulations 155 and 156 exhibited an *ab* phenotype, whereas subpopulation 157 had an *abc* phenotype. Similar observations were made for the Jermy collections.

Measures of genetic variation were also calculated. The mean number of alleles per locus for *I. karstenii* was 2.3; for *I. palmeri* 1.7. The percentage of polymorphic loci for *I. karstenii* was 75%; for *I. palmeri* 50%. A matrix of Nei's (1978) unbiased genetic distance is shown for all populations in Table 3. Cluster analysis using UPGMA was performed on this matrix and the resulting phenogram is shown in Fig. 1. Finally, for *I. karstenii,* genetic distance was plotted against geographic distance between population groups, and a correlation coefficient was calculated (Fig. 2). There is a strong positive correlation between genetic and geographic distance with a correlation coefficient of $r^2=0.775$.

Mean F-statistics were also calculated for *I. karstenii.* The locus *Mdh-2* was removed prior to this analysis because it was scored on a presence/absence basis and therefore could not contribute to measures of heterozygosity. For *I. karstenii* the mean $F_{IS} = -0.060$, the mean $F_{IT} = 0.434$, and the mean $F_{ST} = 0.466$. These values suggest observations on the probable nature of genetic structure within *I. karstenii.* Given the relatively small negative value for F_{IS}, mating within subpopulations is probably near-random. This is not surprising, because *Isoëtes* is heterosporous, producing mega- and microgametophytes from separate spores, with fertilization accomplished by swimming sperm. In

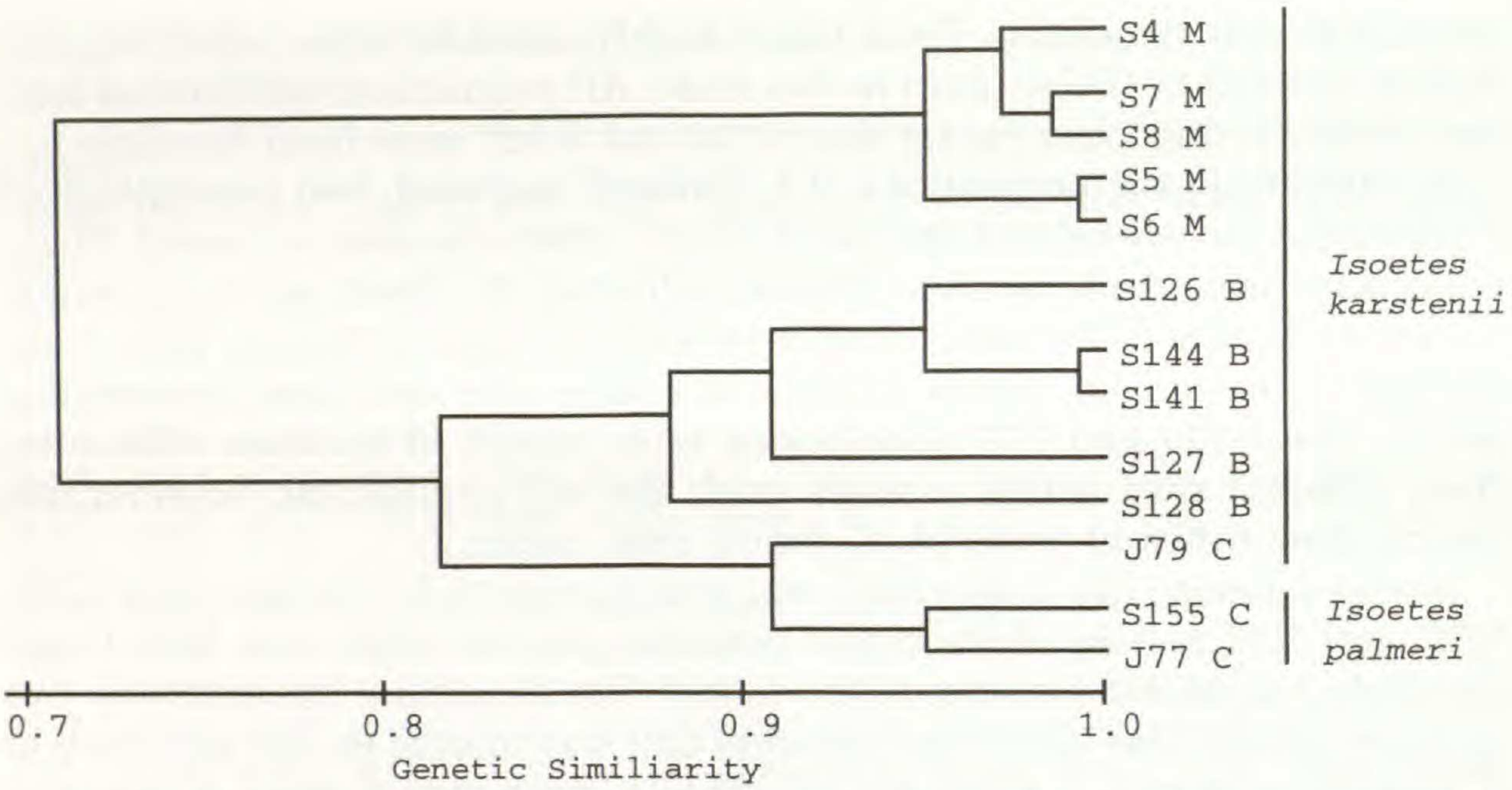

FIG. 1. UPGMA phenogram of population relationships based on Nei's (1978) unbiased genetic distance. Population designations follow Table 1; state of origin is indicated as "M" = Merida, Venezuela; "B" = Boyaca, Colombia; and "C" = Cundinamarca, Colombia.

addition, dispersal of spores is often accomplished via floating leaves that have become detached from the parent plant (or whole plants), yet still contain unbroken sporangia (pers. obs.). As the leaf decays, the spores are released, possibly relatively distant from the parental plant. On the other hand, the relatively high F_{ST} value suggests that mating among subpopulations is not random and that a high degree of differentiation is evident. This observation is further supported by the presence of many unique low-frequency alleles in single populations as well as the high correlation between genetic and geographic distance between populations.

DISCUSSION

Allozyme data have been used for many purposes in systematic studies. In *Isoëtes,* their most common uses have been to provide characters with which to distinguish species and to provide evidence regarding the parentage of allopolyploid taxa or hybrids (e.g. Taylor et al., 1985; Hickey et al., 1989b; Duff and Evans, 1992; Taylor and Hickey, 1992). Generally, fixed allelic differences between taxa at a given locus are used as evidence of reproductive isolation (biological species concept, Hickey et al., 1989b). Additive banding patterns in putative allopolyploid taxa or hybrids at a locus for which the putative parental taxa have fixed differences have been used to provide evidence of the parentage of the allopolyploid or hybrid (Taylor et al., 1985; Hickey et al., 1989b; Taylor and Hickey, 1992).

The results of the analyses of the populations of *I. karstenii* and *I. palmeri* revealed no unique, fixed alleles at any locus that can be used to differentiate these taxa. One locus (*Idh*) exhibited an allele that was unique to *I. palmeri,*

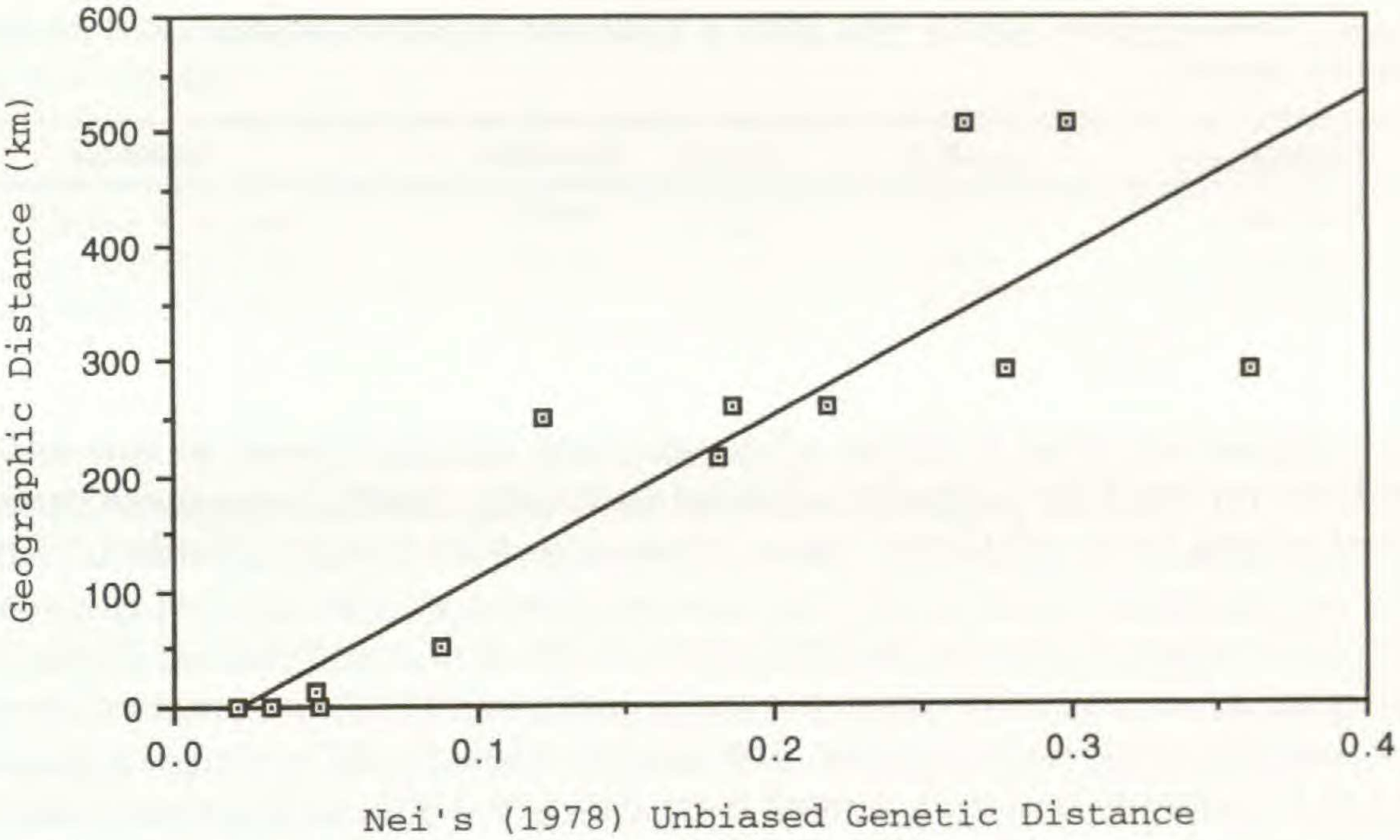

FIG. 2. Scatter plot of geographic vs. Nei's (1978) unbiased genetic distance between groups of populations of *I. karstenii* with line of best fit; $r^2=0.775$.

but not fixed. The fact that these two taxa are distinct species is strongly supported by morphological and cytological evidence (Small, 1994), but the allozyme data shed no light on this hypothesis. Several possibilities exist to explain the apparent lack of genetic divergence between *I. karstenii* and *I. palmeri.* First, this study incorporated a relatively small number of loci (12) and enzyme systems (10). Many authors have discussed the need to examine as many loci as possible (e.g. Archie et al., 1989), and Nei (1978) suggested that 50 or more loci should be examined. Secondly, the appearance of *Isoëtes* in the northern Andean páramos is relatively recent (early Pleistocene; van der Hammen and Cleef, 1986), which suggests that the speciation events that led to these taxa were probably also relatively recent and that there has been relatively little time for genetic divergence. Finally, *I. palmeri* is most likely an allotetraploid, with *I. karstenii* a probable parent (Small, 1994). Therefore half of *I. palmeri*'s nuclear genome is that of *I. karstenii,* and unless there were fixed, unique alleles contributed by the other parent, little or no genetic differentiation would be expected. It is interesting to note that the *I. palmeri* populations, both from Cundinamarca, Colombia, appear most closely related to the *I. karstenii* population that is also from Cundinamarca. Further sampling of *I. palmeri* from throughout its range is necessary to determine whether all *I. palmeri* populations are more closely related to the central Colombian populations of *I. karstenii* or whether multiple origins of the allotetraploid *I. palmeri* have occurred.

The values for genetic identity among populations determined in these analyses were compared with values previously presented in the literature (e.g., Gottlieb, 1977; Soltis and Soltis, 1989; Ranker, 1992). Conspecific populations of both homosporous pteridophytes and seed plants generally have very high

TABLE 4. Unbiased genetic identity (Nei, 1978) in *I. karstenii* by country of origin. N = number of populations sampled.

Country	N	Venezuela	Colombia
Venezuela	5	0.961	
Colombia	6	0.766	0.906

genetic identities (often I >0.95), although mean identity values as low as 0.78 have been reported for some ferns (Soltis and Soltis, 1989). Congeneric species of seed plants have relatively lower values (ca. 0.67 ± 0.07; Gottlieb, 1977), whereas congeneric species of homosporous pteridophytes exhibit an exceedingly wide range of genetic identities (0 to 0.85; Soltis and Soltis, 1989). The mean genetic identity of *I. karstenii* and *I. palmeri* (0.747) is comparable with the values for congeneric species. The genetic identity values among populations of *I. karstenii,* however, ranged from 0.611 to 0.996, with a mean identity of 0.811, a relatively low value that is nonetheless comparable with several homosporous pteridophytes (Soltis and Soltis, 1989). This can be explained, in part, by our sampling. Eleven populations of *I. karstenii* were sampled from a broad geographic range (Edo. Merida, Venezuela, to Depto. Cundinamarca, Colombia, a distance of ca. 600 km) and the resulting mean genetic identity was relatively low.

Furthermore, genetic identity between groups of populations of *I. karstenii* decreases as the distance between them increases. Tables 4 and 5 present mean genetic identity of populations of *I. karstenii* by country and state respectively. The mean identity among the Venezuelan populations, all collected in the state of Merida, was 0.961. Among the Colombian populations, collected in Deptos. Boyaca and Cundinamarca, which are separated by ca. 250 km, the mean genetic identity is lower with a value of 0.906. The mean genetic identity between these two countries is lower still, at 0.766. Further subdivision of the populations into the three states (Merida, Venezuela [northernmost], Boyaca, Colombia [central], and Cundinamarca, Colombia [southernmost]) reinforces the trend (see Table 5). Within the respective states, the mean genetic identity ranges from 0.932 to 0.961 (only a single population was available from Cundinamarca so a comparison could not be made). Genetic identity between Merida and Cundinamarca, the most widely separated locations, was the lowest (0.752), whereas genetic identity between Boyaca (central) and Merida (northern) and Boyaca and Cundinamarca (southern) were 0.769 and 0.855 respectively.

Similar results were found by Duff and Evans (1992) in the species pair *I. caroliniana* and *I. engelmannii.* Although *I. caroliniana* had a high mean interpopulational genetic identity (0.954), *I. engelmannii* had a relatively lower value (0.897). Duff and Evans (1992) cited several possible explanations for the lowered identity values in *I. engelmannii* including its age, low levels of interpopulational gene flow, and interspecific hybridization. Althought any of these factors may be responsible for the low genetic identity of the *I. karstenii*

TABLE 5. Unbiased genetic identity (Nei, 1978) in *I. karstenii* by state of origin. N = number of populations sampled.

State	N	Merida	Boyaca	Cundinamarca
Merida, Venezuela	5	0.961		
Boyaca, Colombia	5	0.769	0.932	
Cundinamarca, Colombia	1	0.752	0.855	no comparison

populations, it would appear that geographic distance and the resulting low levels of interpopulational gene flow have the strongest effects.

The F-statistics provide a measure of genetic differentiation within and between populations. Two conclusions can be drawn from these statistics: 1) intrapopulational genetic variation is high as evidenced by a slightly negative F_{IS}, and 2) interpopulational differentiation is also high as evidenced by the relatively high F_{ST}. That levels of gene flow between *Isoëtes* populations are low has long been assumed, based on levels and patterns of genetic variation (Hickey et al., 1989b; Duff and Evans, 1992; Taylor and Hickey, 1992), and the data presented here provide confirmation of this hypothesis, at least in the case of *I. karstenii*. These conclusions are further supported by data showing the high correlation between genetic and geographic distance (Fig. 2), and can be seen graphically in the phenogram where OTUs are also identified by state of origin (Fig. 1). This is, however, in contrast to the results found by Hickey et al. (1989b) for *I. storkii* T.C. Palmer. Geographic and genetic distance were not well correlated in this species, but probably resulted from local patterns of establishment and extinction of populations in a volatile volcanic and fire-prone habitat.

One final observation also indicates a high degree of interpopulational divergence. Within *I. karstenii* as a whole 75% of the loci are polymorphic, yet within any individual population there is a maximum of 58% polymorphic loci, with a mean of 33% polymorphic loci. This shows that loci that are polymorphic in one population may be monomorphic in another, further indicating population differentiation.

In addition to the calculations of genetic identity and population structure indices, allozymes may provide additional sources of information that can be useful in systematic studies. The occurrence of a post-translational modification product of the locus *Tpi-2* in *Isoëtes* was originally described by Hickey et al. (1989a). In addition to clarifying the interpretation of allozymes at this locus, this phenomenon provided a character of potential phylogenetic utility as it was observed in some, but not all species examined. Specifically, this character-state (presence of the post-translational modification) has been observed in *I. storkii* T.C. Palmer, *I. mexicana* Underw., *I. melanopoda* Gay & Durieu, *I. butleri* Engelm., *I. piedmontana* (N. Pfeiff.) C.F. Reed, *I. melanospora* Engelm., and *I. tegetiformans* Rury, but is absent in *I. cubana* Baker and *I. pallida* Hickey (Hickey et al., 1989a). This study adds two additional species that display this character state, *I. karstenii* and *I. palmeri,* and thereby extends the geographic distribution of this character state to northwestern South Amer-

ica. This serves as a potential link for these northern Andean species with some Central American/Mexican (*I. storkii, I. mexicana*) and North American (*I. melanopoda, I. butleri, I. piedmontana, I. melanospora, I. tegetiformans*) taxa while differentiating them from the Caribbean and southern Mexican species, *I. cubana* (Cuba) and *I. pallida* (Oaxaca, Mexico). Further sampling of this character may add significantly to our knowledge of biogeographic patterns and serve to unite currently fragmented species complexes.

The presence of patterns of apparent fixed heterozygosity in the tetraploid *I. palmeri* was expected, yet the polymorphism of fixed heterozygous patterns was not. Unfortunately, only two samples of *I. palmeri* were surveyed, both from the same general area; formal genetic tests to confirm the "fixation" of heterozygosity were not performed; and only one of the putative parents (*I. karstenii*; Small, 1994) was surveyed. Although hypotheses to explain this phenomenon might be presented (e.g., Gastony, 1990), our sampling is too limited to do so with confidence.

The data presented here confirm and extend results previously obtained regarding the distribution of genetic variation in populations of *Isoëtes* (Hickey et al., 1989b; Duff and Evans, 1992). F-statistics and analyses of geographic vs. genetic distance show that genetic variation in *Isoëtes* can be highly structured, most likely due to the paucity of interpopulational gene flow. Accordingly, these results show that, in studies of genetic variation in *Isoëtes,* it is imperative that sampling be performed from throughout the range in order to observe as much of the variation as possible. This will be especially important in analyses of allopolyploid species, given the possibility of multiple origins of those entities.

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